

**Claims**

1. A variant of a parent protease, comprising a substitution in at least one position of at least one region selected from the group of regions consisting of:

6-18; 22-28; 32-39; 42-58; 62-63; 66-76; 78-100; 103-106; 111-114; 118-131; 134-136; 139-

5 141; 144-151; 155-156; 160-176; 179-181; and 184-188; wherein

(a) the variant has protease activity; and

(b) each position corresponds to a position of amino acids 1 to 188 of SEQ ID NO: 2; and

(c) the variant has a percentage of identity to amino acids 1 to 188 of SEQ ID NO: 2 of at least 60%.

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2. The variant of claim 1 which comprises a substitution in at least one of the following positions: 6; 7; 8; 9; 10; 11; 12; 13; 14; 15; 16; 17; 18; 22; 23; 24; 25; 26; 27; 28; 32; 33; 34; 35; 36; 37; 38; 39; 42; 43; 44; 45; 46; 47; 48; 49; 50; 51; 52; 53; 54; 55; 56; 57; 58; 62; 63; 66; 67; 68; 69; 70; 71; 72; 73; 74; 75; 76; 78; 79; 80; 81; 82; 83; 84; 85; 86; 87; 88; 89; 90; 91; 92; 93; 94; 95; 96; 97; 98; 99; 100; 103; 104; 105; 106; 111; 112; 113; 114; 118; 119; 120; 121; 122; 123; 124; 125; 126; 127; 128; 129; 130; 131; 134; 135; 136; 139; 140; 141; 144; 145; 146; 147; 148; 149; 150; 151; 155; 156; 160; 161; 162; 163; 164; 165; 166; 167; 168; 169; 170; 171; 172; 173; 174; 175; 176; 179; 180; 181; 184; 185; 186; 187; and/or 188.

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3. The variant of claim 2 which comprises a substitution in at least one of the following positions: 6; 7; 8; 9; 10; 12; 13; 16; 17; 18; 22; 23; 24; 25; 26; 27; 28; 32; 33; 37; 38; 39; 42; 43; 44; 45; 46; 47; 48; 49; 50; 51; 52; 53; 54; 55; 56; 58; 62; 63; 66; 67; 68; 69; 70; 71; 72; 73; 74; 75; 76; 78; 79; 80; 81; 82; 83; 84; 85; 86; 87; 88; 89; 90; 91; 92; 93; 94; 95; 96; 97; 98; 99; 100; 103; 105; 106; 111; 113; 114; 118; 120; 122; 124; 125; 127; 129; 130; 131; 134; 135; 136; 139; 140; 141; 144; 145; 146; 147; 148; 149; 150; 151; 155; 156; 160; 161; 162; 163; 164; 165; 166; 167; 168; 169; 170; 171; 172; 173; 174; 175; 176; 179; 180; 181; 184; 185; 186; 187; and/or 188.

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4. The variant of claim 3, which comprises at least one of the following substitutions: 6C; 7P; 8C; 9C; 10E,D; 12E,D; 13E,D,P; 16C; 17C; 18C; 22A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,V,W,Y; 23A,C,D,E,F,G,H,I,K,L,M,P,Q,R,S,T,V,W,Y; 24C,D,E,F,G,H,I,K,L,M,N,P,Q,R,T,V,W,Y; 25C,D,E,F,G,H,I,K,L,M,N,P,Q,R,T,V,W,Y; 26A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y; 27A,C,D,E,F,G,H,I,K,L,M,N,P,R,S,T,V,W,Y; 28A,C,D,E,F,G,H,I,K,L,M,N,Q,R,S,T,V,W,Y; 32C; 33C; 37C; 39R,K; 42E,D; 43A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,W,Y; 44A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,V,W,Y; 45A,C,D,E,F,G,H,K,L,M,N,P,Q,R,S,T,V,W,Y; 46A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y; 47A,C,D,E,F,G,H,I,K,L,M,P,Q,R,S,T,V,W,Y; 48A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y;

49A,C,D,E,F,G,H,I,K,L,M,N,P,S,V,W,Y; 50A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y; 52C;  
 55C; 56R,K; 58E,D; 62C; 63C; 66A,C,D,E,F,G,H,I,K,L,M,N,P,Q,S,T,V,W,Y;  
 67A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y; 68A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,V,W,Y;  
 69A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,T,V,W,Y; 70A,C,D,E,F,G,H,I,K,L,M,P,Q,R,S,T,V,W,Y;  
 5 71A,C,D,E,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y; 72A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,V,W,Y;  
 73A,C,D,E,F,G,H,I,K,M,N,P,Q,R,S,T,V,W,Y; 74A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,V,W,Y;  
 75A,C,D,E,F,G,H,I,K,L,M,P,Q,R,S,T,V,W,Y; 76C; 78A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,T,V,W,Y;  
 79A,C,D,E,F,G,H,I,K,L,M,N,P,Q,S,T,V,W,Y; 80A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W;  
 81A,C,D,E,F,G,H,I,K,L,M,P,Q,R,S,T,V,W,Y; 82A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,V,W,Y;  
 10 83A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y; 84A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y;  
 85A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W; 86C,D,E,F,G,H,I,K,L,M,N,P,R,S,T,V,W,Y;  
 87A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,V,W,Y; 88A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,W,Y;  
 89C,D,E,F,G,H,I,K,L,M,N,P,Q,R,V,W,Y; 90A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y; 92P,R,K;  
 93P; 94C,P; 95E,D; 96E,D,P; 97R,K; 98P; 99R,K; 103C; 105C,P; 106C; 111R,K; 113E,D;  
 15 118R,K; 120E,D; 122K; 124R,K; 125P; 127R,K; 129E,D; 130E,D; 134C; 135P; 136P; 139C;  
 140E,D; 141C; 144C; 145C; 146C; 147W; 148C; 149C; 150E,D; 151P,E,D; 155C; 156C;  
 160A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y; 161A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,T,V,W,Y;  
 162A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y; 163A,C,D,E,F,G,H,I,K,L,M,P,Q,R,S,T,V,W,Y;  
 164A,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y; 165A,C,D,E,F,G,H,I,K,L,M,N,P,Q,T,V,W,Y;  
 20 166A,C,D,E,G,H,I,K,L,M,N,P,Q,R,S,W,Y; 167A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y;  
 168A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y; 169A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,V,W,Y;  
 170A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,V,W,Y; 172C; 173C; 174P; 175P; 176P; 180R,K;  
 181R,K; 184P; 187P; and/or 188R,K.

25 5. The variant of any one of claims 1-4 which comprises at least one of the following pairs  
 of substitutions: 6C+103C; 8C+105C; 76C+85C; 94C+149C; 55C+63C; 16C+145C;  
 33C+144C; 62C+173C; 106C+141C; 9C+17C; 18C+156C; 32C+144C; 37C+52C; 67C+71C;  
 134C+170C; 139C+163C; 146C+148C; and/or 155C+172C.

30 6. The variant of claim 5, which comprises at least one of the following pairs of  
 substitutions: 6C+103C; 8C+105C; 76C+85C; 94C+149C; 55C+63C; 16C+145C; 33C+144C;  
 62C+173C; and/or 106C+141C.

35 7. The variant of any one of claims 1-4 which comprises at least one of the following  
 substitutions: 81P; 82P; 151P; 176P; 24P; 25P; 92P; 93P; 94P; 96P; 98P; 105P; 136P; 184P;  
 187P; 174P; 7P; 13P; 23P; 27P; 125P; 135P; and/or 175P.

8. The variant of claim 7, which comprises at least one of the following substitutions: 81P; 82P; 151P; 176P; 24P; 25P; 92P; 93P; 94P; 96P; 98P; 105P; 136P; 184P; and/or 187P.

5 9. The variant of any one of claims 1-4 which comprises at least one of the following substitutions: 95E,D; 42E,D; 84E,D; 96E,D; 47E,D; 46E,D; 150E,D; 70E,D; 13E,D; 140E,D; 10E,D; 151E,D; 129E,D; 130E,D; 166E,D; 161E,D; 120E,D; 82E,D; 58E,D; 12E,D; 81E,D; 69E,D; 113E,D; 89E,D; and/or 160E,D.

10 10. The variant of claim 9 which comprises at least one of the following substitutions: 95E,D; 42E,D; 84E,D; 96E,D; 47E,D; 46E,D; 150E,D; 70E,D; 13E,D; and/or 140E,D.

15 11. The variant of any one of claims 1-4 which comprises at least one of the following substitutions: 124R,K; 72R,K; 97R,K; 127R,K; 56R,K; 122R,K; 181R,K; 180R,K; 25R,K; 92R,K; 39R,K; 99R,K; 111R,K; 24R,K; 118R,K; 162R,K; and/or 188R,K.

12. The variant of claim 11 which comprises at least one of the following substitutions: 124R,K; 72R,K; 97R,K; 127R,K; 56R,K; 122R,K; 181R,K; 180R,K; 25R,K; and/or 92R,K.

13. The variant of any one of claims 1-4 which comprises at least one of the following substitutions: 147W; 43W.

14. The variant of any one of claims 1-4 which comprises a substitution in at least one position of at least one region selected from the group of regions consisting of:

- (i) 160-170, 78-90, 43-50, 66-75, and 22-28;
- (ii) 160-170, 78-90, 43-50, and 66-75;
- (iii) 160-170, 78-90, and 43-50;
- (iv) 160-170, and 78-90; and/or
- (v) 160-170.

15. The variant of any one of claims 1-4 which comprises at least one of the following substitutions: 6C; 8C; 13E,D; 16C; 24P; 25K,P,R; 33C; 42E,D; 46D,E; 47D,E; 55C; 56R,K; 62C; 63C; 70D,E; 72K,R; 76C; 81P; 82P; 84D,E; 85C; 92P,R,K; 93P; 94C,P; 95E,D; 96E,D,P; 97R,K; 98P; 103C; 105C,P; 106C; 122R,K; 124R,K; 127R,K; 136P; 140E,D; 141C; 144C; 145C; 149C; 150E,D; 151P; 173C; 176P; 180R,K; 181R,K; 184P; and/or 187P; preferably 47D, 49K, 92K, 127R, and/or 166A.

16. The variant of any one of claims 1-3, which comprises at least one of the following

substitutions: G6C; L7P; A8C; Y9C; T10E,D,Y; G12E,D; G13E,D,P; S16C; V17C; G18C; T22A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,V,W,Y; N23A,C,D,E,F,G,H,I,K,L,M,P,Q,R,S,T,V,W,Y; A24C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y (preferably A24S); A25C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y (preferably A25S);

5 G26A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y; Q27A,C,D,E,F,G,H,I,K,L,M,N,P,R,S,T,V,W,Y; P28A,C,D,E,F,G,H,I,K,L,M,N,Q,R,S,T,V,W,Y; T32C; A33C; G37C; R38T; V39R,K; Q42E,D,G,P; V43A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,W,Y; T44A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,V,W,Y (preferably T44S); I45A,C,D,E,F,G,H,K,L,M,N,P,Q,R,S,T,V,W,Y; G46A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y;

10 N47A,C,D,E,F,G,H,I,K,L,M,P,Q,R,S,T,V,W,Y; G48A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y; R49A,C,D,E,F,G,H,I,K,L,M,N,P,Q,S,T,V,W,Y (preferably R49T,Q); G50A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y; V51T; F52C; E53Q; Q54N,R; S55C; V56I,R,K; P58E,D; A62C,S; A63C; R66A,C,D,E,F,G,H,I,K,L,M,N,P,Q,S,T,V,W,Y; G67A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y; T68A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,V,W,Y;

15 S69A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,T,V,W,Y; N70A,C,D,E,F,G,H,I,K,L,M,P,Q,R,S,T,V,W,Y; F71A,C,D,E,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y; T72A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,V,W,Y; L73A,C,D,E,F,G,H,I,K,M,N,P,Q,R,S,T,V,W,Y; T74A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,V,W,Y; N75A,C,D,E,F,G,H,I,K,L,M,P,Q,R,S,T,V,W,Y; L76C; S78A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,T,V,W,Y; R79A,C,D,E,F,G,H,I,K,L,M,N,P,Q,S,T,V,W,Y;

20 Y80A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W; N81A,C,D,E,F,G,H,I,K,L,M,P,Q,R,S,T,V,W,Y; T82A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,V,W,Y (preferably T82S); G83A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y; G84A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y; Y85A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W; A86C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y (preferably A86Q); T87A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,V,W,Y (preferably T87S);

25 V88A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,W,Y; A89C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y (preferably A89T,S); G90A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y; H91T,S; N92P,R,K,S; Q93P; A94C,P; P95A,E,D; I96A,E,D,P; G97R,K; S98P; S99A,Q,R,K; V100I; S103C; S105C,P; T106C; C111R,K; T113E,D; I114V; G118N,R,K; S120T,E,D; S122R,K; P124R,K; E125P,Q; T127R,K; T129E,D,Y,Q; N130E,D,S; M131L; T134C; T135P,N; V136P; E139C; P140E,D;

30 G141C; G144C; G145C; S146C; Y147F,W; I148C; S149C; G150E,D; T151P,E,D,S; G155C; V156C; G160A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y; S161A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,T,V,W,Y; G162A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y; N163A,C,D,E,F,G,H,I,K,L,M,P,Q,R,S,T,V,W,Y; C164A,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y; R165A,C,D,E,F,G,H,I,K,L,M,N,P,Q,S,T,V,W,Y (preferably R165S);

35 T166A,C,D,F,E,G,H,I,K,L,M,N,P,Q,R,S,V,W,Y (preferably T166V,F); G167A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y; G168A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y; T169A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,V,W,Y; T170A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,V,W,Y;

F171Y; Y172C; Q173C; E174P; V175P; T176N,P; V179I,L; N180R,K,S; S181R,K; V184L,P;  
 R185T; L186I; R187P; and/or T188R,K; preferably at least one of the following:  
 T10Y, A25S, R38T, Q42P, T44S, N47D, R49K, Q54R, V56I, A62S, T82S, N92K, S99A,  
 G118N, S120T, S122R, E125Q, T127R, T129Y, N130S, M131L, R165S, T166A, F171Y,  
 5 T176N, V179L, N180S, V184L, and/or R185T.

17. The variant of any one of claims 1-3, which comprises at least one of the following substitutions: G6C; L7P; A8C; Y9C; Y10E,D,T; G12E,D; G13E,D,P; S16C; V17C; G18C;  
 T22A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,V,W,Y; N23A,C,D,E,F,G,H,I,K,L,M,P,Q,R,S,T,V,W,Y;  
 10 S24A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,T,V,W,Y (preferably S24A);  
 A25C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y (preferably A25S);  
 G26A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y; Q27A,C,D,E,F,G,H,I,K,L,M,N,P,R,S,T,V,W,Y;  
 P28A,C,D,E,F,G,H,I,K,L,M,N,Q,R,S,T,V,W,Y; T32C; A33C; G37C; T38R; V39R,K;  
 G42E,D,P,Q; V43A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,W,Y;  
 15 T44A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,V,W,Y (preferably T44S);  
 I45A,C,D,E,F,G,H,K,L,M,N,P,Q,R,S,T,V,W,Y; G46A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y;  
 N47A,C,D,E,F,G,H,I,K,L,M,P,Q,R,S,T,V,W,Y; G48A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y;  
 T49A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,V,W,Y (preferably T49R,Q);  
 G50A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y; T51V; F52C; Q53E; N54Q,R; S55C; V56I,R,K;  
 20 P58E,D; A62C,S; A63C; R66A,C,D,E,F,G,H,I,K,L,M,N,P,Q,S,T,V,W,Y;  
 G67A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y; T68A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,V,W,Y;  
 S69A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,T,V,W,Y; N70A,C,D,E,F,G,H,I,K,L,M,P,Q,R,S,T,V,W,Y;  
 F71A,C,D,E,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y; T72A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,V,W,Y;  
 L73A,C,D,E,F,G,H,I,K,M,N,P,Q,R,S,T,V,W,Y; T74A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,V,W,Y;  
 25 N75A,C,D,E,F,G,H,I,K,L,M,P,Q,R,S,T,V,W,Y; L76C;  
 S78A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,T,V,W,Y; R79A,C,D,E,F,G,H,I,K,L,M,N,P,Q,S,T,V,W,Y;  
 Y80A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W; N81A,C,D,E,F,G,H,I,K,L,M,P,Q,R,S,T,V,W,Y;  
 S82A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,T,V,W,Y (preferably S82T);  
 G83A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y; G84A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y;  
 30 Y85A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W; Q86A,C,D,E,F,G,H,I,K,L,M,N,P,R,S,T,V,W,Y  
 (preferably Q86A); S87A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,T,V,W,Y (preferably S87T);  
 V88A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,W,Y; T89A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,V,W,Y  
 (preferably T89A,S); G90A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y; T91H,S; S92P,R,K,N;  
 Q93P; A94C,P; P95A,E,D; A96E,D,I,P; G97R,K; S98P; A99R,K,S; V100I; S103C; S105C,P;  
 35 T106C; C111R,K; T113E,D; I114V; N118G,R,K; T120E,D,S; R122K,S; P124R,K; Q125E,P;  
 T127R,K; Y129E,D,T; S130E,D,N; L131M; T134C; N135P,T; V136P; E139C; P140E,D;  
 G141C; G144C; G145C; S146C; F147W,Y; I148C; S149C; G150E,D; S151P,E,D,T; G155C;

V156C; G160A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y;  
 S161A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,T,V,W,Y; G162A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y;  
 N163A,C,D,E,F,G,H,I,K,L,M,P,Q,R,S,T,V,W,Y; C164A,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y;  
 S165A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,T,V,W,Y (preferably S165R);  
 5 V166A,C,D,E,G,H,I,K,L,M,N,P,Q,R,S,T,W,Y (preferably V166F,T);  
 G167A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y; G168A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y;  
 T169A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,V,W,Y; T170A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,V,W,Y;  
 Y171F; Y172C; Q173C; E174P; V175P; T176P,N; I179L; V N180R,K,S; S181R,K; V184L,P;  
 R185T; I186L; R187P; and/or T188R,K; preferably at least one of the following:  
 10 S24A, A25S, G42P, T44S, N47D, T49K, T51V, Q53E, N54R, V56I, A62S, Q86A, S87T, T89A,  
 T91H, S92N, S92K, A96I, T127R, N135T, F147Y, S151T, V166A, T176N, I179L, N180S,  
 V184L, R185T, and/or I186L.

18. The variant of any one of claims 1-3, which comprises at least one of the following  
 15 substitutions: G6C; L7P; A8C; Y9C; T10E,D,Y; G12E,D,P; S16C; V17C; G18C;  
 T22A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,V,W,Y; N23A,C,D,E,F,G,H,I,K,L,M,P,Q,R,S,T,V,W,Y;  
 A24C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y (preferably A24S);  
 A25C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y (preferably A25S);  
 G26A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y; Q27A,C,D,E,F,G,H,I,K,L,M,N,P,R,S,T,V,W,Y;  
 20 P28A,C,D,E,F,G,H,I,K,L,M,N,Q,R,S,T,V,W,Y; T32C; A33C; G37C; R38T; V39R,K;  
 Q42E,D,G,P; V43A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,W,Y;  
 S44A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,T,V,W,Y (preferably S44T);  
 I45A,C,D,E,F,G,H,K,L,M,N,P,Q,R,S,T,V,W,Y; G46A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y;  
 N47A,C,D,E,F,G,H,I,K,L,M,P,Q,R,S,T,V,W,Y; G48A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y;  
 25 Q49A,C,D,E,F,G,H,I,K,L,M,N,P,R,S,T,V,W,Y (preferably Q49R,T);  
 G50A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y; V51T; F52C; E53Q; Q54N,R; S55C; I56R,K;  
 P58E,D; A62C,S; A63C; R66A,C,D,E,F,G,H,I,K,L,M,N,P,Q,S,T,V,W,Y;  
 G67A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y; T68A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,V,W,Y;  
 S69A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,T,V,W,Y; N70A,C,D,E,F,G,H,I,K,L,M,P,Q,R,S,T,V,W,Y;  
 30 F71A,C,D,E,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y; T72A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,V,W,Y;  
 L73A,C,D,E,F,G,H,I,K,M,N,P,Q,R,S,T,V,W,Y; T74A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,V,W,Y;  
 N75A,C,D,E,F,G,H,I,K,L,M,P,Q,R,S,T,V,W,Y; L76C;  
 S78A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,T,V,W,Y; R79A,C,D,E,F,G,H,I,K,L,M,N,P,Q,S,T,V,W,Y;  
 Y80A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W; N81A,C,D,E,F,G,H,I,K,L,M,P,Q,R,S,T,V,W,Y;  
 35 T82A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,V,W,Y (preferably T82S);  
 G83A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y; G84A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y;  
 Y85A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W; A86C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y

- (preferably A86Q); T87A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,V,W,Y (preferably T87S); V88A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,W,Y; A89C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y (preferably A89T,S); G90A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y; H91T,S; N92P,R,K,S; Q93P; A94C,P; P95A,E,D; I96A,E,D,P; G97R,K; S98P; S99A,Q,R,K; V100I; S103C; S105C,P;
- 5 T106C; C111R,K; T113E,D; I114V; G118N,R,K; S120E,D,T; S122R,K; P124R,K; E125P,Q; T127R,K; T129E,D,Q,Y; N130E,D,S; M131L; T134C; T135N,P; V136P; E139C; P140E,D; G141C; G144C; G145C; S146C; Y147F,W; I148C; S149C; G150E,D; N151P,E,D,T; G155C; V156C; G160A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y;
- 10 S161A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,T,V,W,Y; G162A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y; N163A,C,D,E,F,G,H,I,K,L,M,P,Q,R,S,T,V,W,Y; C164A,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y; R165A,C,D,E,F,G,H,I,K,L,M,N,P,Q,S,T,V,W,Y (preferably R165S);
- T166A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,V,W,Y (preferably T166F,V); G167A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y; G168A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y; T169A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,V,W,Y; T170A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,V,W,Y;
- 15 F171Y; Y172C; Q173C; E174P; V175P; T176N,P; V179I,L; N180R,K,S; S181R,K; V184L,P; R185T; L186I; R187P; and/or T188R,K; preferably at least one of the following: T10Y, A25S, R38T, Q42P, N47D, Q49K, Q54R, A62S, T82S, N92K, S99A, G118N, S120T, S122R, E125Q, T127R, T129Y, N130S, M131L, N151T, R165S, T166A, F171Y, T176N, V179L, N180S, V184L, and/or R185T.
- 20
19. The variant of any one of claims 1-3, which comprises at least one of the following substitutions: G6C; L7P; A8C; Y9C; T10E,D,Y; G12E,D; G13E,D,P; S16C; V17C; G18C; T22A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,V,W,Y; N23A,C,D,E,F,G,H,I,K,L,M,P,Q,R,S,T,V,W,Y; A24C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y (preferably A24S);
- 25 A25C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y (preferably A25S); G26A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y; Q27A,C,D,E,F,G,H,I,K,L,M,N,P,R,S,T,V,W,Y; P28A,C,D,E,F,G,H,I,K,L,M,N,Q,R,S,T,V,W,Y; T32C; A33C; G37C; R38T; V39R,K; Q42E,D,G,P; V43A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,W,Y; T44A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,V,W,Y (preferably T44S);
- 30 I45A,C,D,E,F,G,H,K,L,M,N,P,Q,R,S,T,V,W,Y; G46A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y; N47A,C,D,E,F,G,H,I,K,L,M,P,Q,R,S,T,V,W,Y; G48A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y; R49A,C,D,E,F,G,H,I,K,L,M,N,P,Q,S,T,V,W,Y (preferably R49Q,T); G50A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y; V51T; F52C; E53Q; Q54N,R; S55C; I56R,K; P58E,D; A62C,S; A63C; R66A,C,D,E,F,G,H,I,K,L,M,N,P,Q,S,T,V,W,Y;
- 35 G67A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y; T68A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,V,W,Y; S69A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,T,V,W,Y; N70A,C,D,E,F,G,H,I,K,L,M,P,Q,R,S,T,V,W,Y; F71A,C,D,E,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y; T72A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,V,W,Y;

L73A,C,D,E,F,G,H,I,K,M,N,P,Q,R,S,T,V,W,Y; T74A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,V,W,Y;  
 N75A,C,D,E,F,G,H,I,K,L,M,P,Q,R,S,T,V,W,Y; L76C;  
 S78A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,T,V,W,Y; R79A,C,D,E,F,G,H,I,K,L,M,N,P,Q,S,T,V,W,Y;  
 Y80A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W; N81A,C,D,E,F,G,H,I,K,L,M,P,Q,R,S,T,V,W,Y;  
 5 T82A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,V,W,Y (preferably T82S);  
 G83A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y; G84A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y;  
 Y85A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W; A86C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y  
 (preferably A86Q); T87A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,V,W,Y (preferably T87S);  
 V88A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,W,Y; A89C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y  
 10 (preferably A89S,T); G90A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y; H91T,S; N92P,R,K,S;  
 Q93P; A94C,P; P95A,E,D; I96A,E,D,P; G97R,K; S98P; S99A,Q,R,K; V100I; S103C; S105C,P;  
 T106C; C111R,K; T113E,D; I114V; G118N,R,K; S120E,D,T; S122R,K; P124R,K; E125P,Q;  
 T127R,K; T129E,D,Q,Y; N130E,D,S; M131L; T134C; T135N,P; V136P; E139C; P140E,D;  
 G141C; G144C; G145C; S146C; Y147F,W; I148C; S149C; G150E,D; N151P,E,D,S,T;  
 15 G155C; V156C; G160A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y;  
 S161A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,T,V,W,Y; G162A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y;  
 N163A,C,D,E,F,G,H,I,K,L,M,P,Q,R,S,T,V,W,Y; C164A,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y;  
 R165A,C,D,E,F,G,H,I,K,L,M,N,P,Q,S,T,V,W,Y (preferably R165S);  
 T166A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,V,W,Y (preferably T166V);  
 20 G167A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y; G168A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y;  
 T169A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,V,W,Y; T170A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,V,W,Y;  
 F171Y; Y172C; Q173C; E174P; V175P; T176N,P; V179I,L; N180R,K,S; S181R,K; V184L,P;  
 R185T; L186I; R187P; and/or T188R,K; preferably at least one of the following:  
 T10Y, A25S, R38T, G42P, T44S, N47D, R49K, Q54R, A62S, T82S, N92K, S99A, G118N,  
 25 S120T, S122R, E125Q, T127R, T129Y, N130S, M131L, N151T, R165S, T166A, F171Y,  
 T176N, V179L, N180S, V184L, and/or R185T.

20. The variant of any one of claims 1-3, which comprises at least one of the following substitutions: G6C; L7P; A8C; Y9C; T10E,D,Y; G12E,D; G13E,D,P; S16C; V17C; G18C;  
 30 T22A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,V,W,Y; N23A,C,D,E,F,G,H,I,K,L,M,P,Q,R,S,T,V,W,Y;  
 A24C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y (preferably A24S);  
 S25A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,T,V,W,Y (preferably S25A);  
 G26A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y; Q27A,C,D,E,F,G,H,I,K,L,M,N,P,R,S,T,V,W,Y;  
 P28A,C,D,E,F,G,H,I,K,L,M,N,Q,R,S,T,V,W,Y; T32C; A33C; G37C; T38R; V39R,K;  
 35 P42E,D,G,Q; V43A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,W,Y;  
 S44A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,T,V,W,Y (preferably S44T);  
 I45A,C,D,E,F,G,H,K,L,M,N,P,Q,R,S,T,V,W,Y; G46A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y;

N47A,C,D,E,F,G,H,I,K,L,M,P,Q,R,S,T,V,W,Y; G48A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y;  
 Q49A,C,D,E,F,G,H,I,K,L,M,N,P,R,S,T,V,W,Y (preferably Q49R,T);  
 G50A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y; V51T; F52C; E53Q; R54N,Q; S55C; V56I,R,K;  
 P58E,D; S62A,C; A63C; R66A,C,D,E,F,G,H,I,K,L,M,N,P,Q,S,T,V,W,Y;

5 G67A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y; T68A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,V,W,Y;  
 S69A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,T,V,W,Y; N70A,C,D,E,F,G,H,I,K,L,M,P,Q,R,S,T,V,W,Y;  
 F71A,C,D,E,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y; T72A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,V,W,Y;  
 L73A,C,D,E,F,G,H,I,K,M,N,P,Q,R,S,T,V,W,Y; T74A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,V,W,Y;  
 N75A,C,D,E,F,G,H,I,K,L,M,P,Q,R,S,T,V,W,Y; L76C;

10 S78A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,T,V,W,Y; R79A,C,D,E,F,G,H,I,K,L,M,N,P,Q,S,T,V,W,Y;  
 Y80A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W; N81A,C,D,E,F,G,H,I,K,L,M,P,Q,R,S,T,V,W,Y;  
 T82A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,V,W,Y (preferably T82S);  
 G83A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y; G84A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y;  
 Y85A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W; A86C,D,E,F,G,H,I,K,L,M,N,P,R,Q,S,T,V,W,Y  
 15 (preferably A86Q); T87A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,V,W,Y (preferably T87S);  
 V88A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,W,Y; S89A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,T,V,W,Y  
 (preferably S89A,T); G90A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y; S91H,T; S92P,R,K,N;  
 Q93P; A94C,P; A95E,D,P; I96A,E,D,P; G97R,K; S98P; Q99A,R,K,S; I100V; S103C; S105C,P;  
 T106C; C111R,K; T113E,D; V114I; G118N,R,K; T120E,D,S; S122R,K; P124R,K; Q125E,P;  
 20 T127R,K; Q129E,D,Y,T; N130E,D,S; L131M; T134C; N135P,T; V136P; E139C; P140E,D;  
 G141C; G144C; G145C; S146C; F147W,Y; I148C; S149C; G150E,D; S151P,E,D,T; G155C;  
 V156C; G160A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y;  
 S161A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,T,V,W,Y; G162A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y;  
 N163A,C,D,E,F,G,H,I,K,L,M,P,Q,R,S,T,V,W,Y; C164A,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y;  
 25 S165A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,T,V,W,Y (preferably S165R);  
 F166A,C,D,E,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y (preferably F166,T,V);  
 G167A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y; G168A,C,D,E,F,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y;  
 T169A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,V,W,Y; T170A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,V,W,Y;  
 Y171F; Y172C; Q173C; E174P; V175P; T176N,P; L179I,V; S180R,K,N; S181R,K; L184P,V;  
 30 T185R; L186I; R187P; and/or T188R,K; preferably at least one of the following:  
 T10Y, N47D, Q49K, V56I, T82S, S89A, S91H, S92N, S92K, A95P, Q99A, I100V, V114I,  
 G118N, S122R, T127R, Q129Y, N130S, N135T, F147Y, S151T, and/or F166A.

35 21. An isolated nucleic acid sequence comprising a nucleic acid sequence which encodes  
 the protease variant of any one of claims 1-20.

22. A nucleic acid construct comprising the nucleic acid sequence of claim 21 operably

linked to one or more control sequences that direct the production of the protease variant in a suitable expression host.

23. A recombinant expression vector comprising the nucleic acid construct of claim 22.

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24. A recombinant host cell comprising the nucleic acid construct of claim 22 and/or the expression vector of claim 23.

25. A method for producing the protease variant of any one of claims 1-20, the method comprising:

10 (a) cultivating the host cell of claim 24 to produce a supernatant comprising the variant; and  
(b) recovering the variant.

15 26. A transgenic plant, or plant part, capable of expressing a protease variant of any one of claims 1-20.

27. A transgenic, non-human animal, or products, or elements thereof, being capable of expressing a protease variant of any one of claims 1-20.

20 28. An animal feed additive comprising at least one protease variant of any one of claims 1-20, and

25 (a) at least one fat soluble vitamin;  
(b) at least one water soluble vitamin; and/or  
(c) at least one trace mineral.

29. An animal feed composition having a crude protein content of 50 to 800 g/kg and comprising the protease variant of any one of claims 1-20.

30. A method for improving the nutritional value of an animal feed, wherein the protease variant of any one of claims 1-20, and/or the composition of any one of claims 28 or 29 is added to the feed.

35 31. A method for the treatment of proteins, comprising the step of adding the protease variant of any one of claims 1-20, and/or the composition of any one of claims 28-29 to at least one protein or protein source.

32. Use of the protease variant of any one of claims 1-20, and/or the composition of any

one of claims 28-29 (i) in animal feed; (ii) in the preparation of animal feed; (iii) for improving the nutritional value of animal feed; and/or (iv) for the treatment of proteins.

5      33.     Use of the protease variant of any one of claims 1-20 in detergents.

34.    A protease having a 3D structure which is i) superimposable with, ii) similar to, iii) substantially similar to, or iv) identical to the structure provided by the coordinates of the 3D structure of Fig. 2.

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35.    A 3D structure comprising, or consisting of, the structure provided by the coordinates of Fig. 2.

36.    The 3D structure of claim 35 which has protease activity.